# APPENDIX HH:

## Michigan CIWPIS Data and Wetland Study Information

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Wednesday, November 23, 2011

# CIWPIS on Line

Notices and Hearings
New Applications

## Coastal and Inland Waters Permit Information System

17 Records returned.
Click on Folder icon for specific information.

**Search For:**

Year: Year
Sort by: Date D
County: Baraga
Township Name:
Twn: 51n Rng: 33w Sec:
Waterbody:
File Number:
Applicant Name:

Search

| File No: | Name: | Status: |
|---|---|---|
| 11-07-0011-P | Pettibone Michigan Corp LLC | Permit Issued |
| 11-07-0008-P | Village of Baraga | Permit Issued |
| 11-07-0007-P | Village of Baraga | Permit Issued |
| 11-07-0003-P | Village of Baraga | Permit Issued |
| 10-07-0046-P | Keweenaw Bay Indian Community | Closed Lack of Information |
| 10-07-0020-P | Village of Baraga | Permit Issued |
| 10-07-0018-P | Baraga County Road Commission | Permit Issued |
| 09-07-0019-P | Robert V Ross | Permit Issued |
| 08-07-0053-P | Baraga County Road Commission | Permit Issued After the Fact |
| 08-07-0039-P | Paul Getzen | Permit Issued |
| 07-07-0043-P | Village of Baraga | Permit Issued |
| 07-07-0022-P | Village of Baraga | Permit Issued |
| 07-07-0012-P | Village of Baraga | Permit Issued |
| 07-07-0007-P | Village of L'Anse | Closed Lack of Information |
| 06-07-0018-P | David Coponen | Denied |
| 06-07-0012-P | Baraga County Road Commission | Permit Issued |
| 06-07-0003-P | U.P. Timber Company | Permit Issued Modified by Staff |

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Fill in any or all of the fields above and press the Search button. Placing your cursor over the field will display tool tips. If this site does not work correctly please let us know.

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**Wednesday, November 23, 2011**

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Notices and Hearings

New Applications

## Coastal and Inland Waters Permit Information System

10 Records returned.

Click on Folder icon for specific information.

| File No: | Name: | Status: |
|---|---|---|
| 10-07-0049-P | Baraga County Road Commission | Closed Duplicate File |
| 10-07-0048-P | Baraga County Road Commission | Permit Issued |
| 10-07-0040-P | Plum Creek Timberlands | Permit Issued |
| 10-07-0011-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 09-07-0009-P | Rose Mary Haataja | Permit Issued |
| 08-07-0007-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 07-07-0023-P | Patrick Newland | Permit Issued |
| 06-07-0024-P | Baraga County Road Commission | Permit Issued |
| 06-07-0011-P | Baraga County Road Commission | Permit Issued |
| 06-07-0004-P | Plum Creek | Permit Issued |

**Search For:**

Year: Year

Sort by: Date D

County: Baraga

Township Name:

Twn: 51n Rng: 32w Sec:

Waterbody:

File Number:

Applicant Name:

Search

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Wednesday, November 23, 2011

# CIWPIS on Line

Notices and Hearings

New Applications

## Coastal and Inland Waters Permit Information System

32 Records returned.
Click on Folder icon for specific information.

Search For:

Year: Year
Sort by: Date D
County: Baraga
Township Name:
Twn: 50n Rng: 32w Sec:
Waterbody:
File Number:
Applicant Name:

Search

Help:
Fill in any or all of the fields above and press the Search button. Placing your cursor over the field will display tool tips. If this site does not work correctly please let us know.

| File No: | Name: | Status: |
|---|---|---|
| 11-07-0033-P | Plum Creek | Permit Issued |
| 11-07-0032-P | Baraga County Road Commission | Permit Issued |
| 10-07-0037-P | Plum Creek | Permit Issued |
| 10-07-0012-P | Plum Creek | Permit Issued |
| 10-07-0013-P | William "Sam" Morrow | Permit Issued |
| 09-07-0026-P | Plum Creek | Permit Issued |
| 09-07-0027-P | Plum Creek | Permit Issued Modified by Staff |
| 09-07-0021-P | Plum Creek | Permit Issued |
| 09-07-0022-P | Plum Creek | Permit Issued |
| 09-07-0023-P | Plum Creek | Permit Issued |
| 08-07-0044-P | Plum Creek | Permit Issued |
| 08-07-0045-P | Plum Creek | Permit Issued |
| 08-07-0046-P | Plum Creek | Permit Issued |
| 08-07-0047-P | Plum Creek | Permit Issued |
| 08-07-0048-P | Plum Creek | Permit Issued |
| 08-07-0010-P | Plum Creek Timber | Permit Issued |
| 08-07-0011-P | Plum Creek Timber | Permit Issued |
| 08-07-0008-P | Plum Creek | Permit Issued |
| 08-07-0009-P | Plum Creek | Permit Issued |
| 08-07-0005-P | American Forest Management | Permit Issued |
| 07-07-0044-P | American Forest Management | Permit Issued |
| 07-07-0045-P | American Forest Management | Permit Issued |
| 07-07-0046-P | American Forest Management | Permit Issued |
| 07-07-0038-P | Plum Creek | Permit Issued |
| 07-07-0037-P | Plum Creek | Permit Issued |
| 07-07-0024-P | Plum Creek | Permit Issued |

| | | |
|---|---|---|
| 06-07-0048-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 06-07-0031-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 06-07-0032-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 06-07-0033-P | Plum Creek Timberlands, L.P. | Permit Issued |
| 06-07-0022-P | Baraga County Road Commission | Permit Issued |
| 06-07-0016-P | All-Wood, Inc. | Permit Issued |

Wednesday, November 23, 2011

# CIWPIS on Line

Notices and Hearings
New Applications

## Coastal and Inland Waters Permit Information System

**Search For:**

Year: Year
Sort by: Date D
County: Baraga
Township Name:
Twn: 50n Rng: 33w Sec:
Waterbody:
File Number:
Applicant Name:

Search

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26 Records returned.
Click on Folder icon for specific information.

| File No: | Name: | Status: |
|---|---|---|
| 11-07-0034-P | L'Anse Warden Electric Company, LLC | Assigned to Field Reviewer |
| 10-07-0035-P | L'Anse Warden Electric Co | Permit Issued |
| 10-07-0043-P | L'Anse Warden Electric Company | Closed Duplicate File |
| 10-07-0034-P | Baraga County Road Commission | Permit Issued |
| 10-07-0027-P | Michigan Dept. of Transporation | Permit Issued |
| 10-07-0024-P | CertainTeed Corporation | Permit Issued |
| 10-07-0003-P | Michigan Dept. of Transportation | Permit Issued |
| 10-07-0002-P | Village of L'Anse | Permit Issued |
| 10-07-0001-P | Village of L'Anse | Permit Issued |
| 09-07-0041-P | Semco Energy Natural Gas Company | Permit Issued |
| 09-07-0034-P | Kahkonen Excavating, Inc. | Permit Issued |
| 09-07-0029-P | Village of L'Anse | Permit Issued Conditional |
| 09-07-0016-P | Baraga County Road Commission | Permit Issued |
| 09-07-0010-P | Baraga County Memorial Hospital | Permit Issued |
| 08-07-0052-P | Baraga County Memorial Hospital | Permit Issued |
| 09-07-0005-P | Baraga County Road Commission | Permit Issued Revised per Applicant Request |
| 08-07-0034-P | Michigan Dept. of Transportation | Permit Issued |
| 08-07-0033-P | Baraga County Road Commission | Permit Issued |
| 08-07-0028-P | Patrick T. Coady | Permit Issued |
| 07-07-0052-P | Village of L'Anse | Permit Issued |
| 07-07-0050-P | Baraga County Road Commission | Permit Issued After the Fact |
| 07-07-0042-P | Certainteed Gypsum and Ceilings | Permit Issued |
| 07-07-0041-P | L'Anse Warden Electric Company LLC | Permit Issued |
| 06-07-0041-P | Baraga County Tourist & Rec. Assoc. | Permit Issued |

| | | |
|---|---|---|
| 06-07-0019-P | Michigan Dept. of Transportation | Permit Issued |
| 06-07-0013-P | L'Anse Township | Permit Issued |

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Wednesday, November 23, 2011

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## Coastal and Inland Waters Permit Information System

Notices and Hearings

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10-07-0011-P

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**Note:** Some fields are blank dependent upon application type and/or status.

**Upper Peninsula District Office**
420 Fifth Street
Gwinn , Michigan
49841-3004
906-346-8300

**File No:** 10-07-0011-P **Status:** Permit Issued

| | | | |
|---|---|---|---|
| Name: | Plum Creek Timberlands, L.P. | County: | Baraga |
| Address: | 15800 Mead Road<br>L'Anse, MI 49946 | Twn/Rng/Sec: | 51N/32W/23 |
| | | Government: | L'Anse Township |
| | | Subdivision: | |
| Waterbody: | Tributaries to Silver River | Lot: | |
| ProjectName: | Mount Ervast | Tax ID: | |

Activity: Culverts MiTAPS:
Activity:
Activity:

| Parts: | 301 | 325 | 303 | 31 | 315 | 323 | 353 | Sec404 |
|---|---|---|---|---|---|---|---|---|
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|---|---|---|---|
| Type: | Minor Project | Field: | Sheila B. Meier |
| Renewal: | | Entry: | |

| | | | |
|---|---|---|---|
| Date Received: | 4/26/2010 | Date Final Action: | 5/26/2010 |
| Date Sent to Field: | | Date Permit Expires: | 5/25/2015 |
| Date Extended: | | Date Revised: | |
| Date Site Inspection: | | Date Public Notice: | |
| Date Public Hearing: | | Date CR Mailed: | |

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Environmental Toxicology and Chemistry, Vol. 30, No. 7, pp. 1637–1641, 2011

Printed in the USA
DOI: 10.1002/etc.544

# EFFECTS OF SIX CHEMICAL DEICERS ON LARVAL WOOD FROGS (*RANA SYLVATICA*)

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†Michigan Technological University, Houghton, Michigan, USA
‡Pennsylvania State University, University Park, Pennsylvania, USA

(*Submitted* 2 *November* 2010; *Returned for Revision* 17 *December* 2010; *Accepted* 14 *March* 2011)

**Abstract**—Widespread and intensive application of road deicers, primarily road salt (NaCl), in North America threatens water quality and the health of freshwater ecosystems. Intensive use of NaCl can be harmful to sensitive members of freshwater ecosystems such as amphibians. Detection of negative effects of NaCl application has prompted the search for alternative chemical deicers with lower environmental impacts. We conducted a series of 96-h acute toxicity tests to determine the negative sensitivity of larval wood frogs (*Rana* [*Lithobates*] *sylvatica*) to six deicing chemicals: urea ($CH_4N_2O$), sodium chloride (NaCl), magnesium chloride ($MgCl_2$), potassium acetate ($CH_3COOK$), calcium chloride ($CaCl_2$), and calcium magnesium acetate ($C_8H_{12}CaMgO_8$). Acetates are sometimes touted as environmentally friendly alternatives to NaCl but have not been examined in enough detail to warrant this designation. When exposed to a range of environmentally realistic concentrations of these chemicals, larvae were least sensitive (i.e., had the lowest mortality rate) to $CH_4N_2O$, NaCl, and $MgCl_2$ and most sensitive to acetates ($C_8H_{12}CaMgO_8$, $CH_3COOK$) and $CaCl_2$. Our observed median lethal concentration estimates ($LC50_{96\text{-h}}$) for NaCl were over two times higher than values presented in previous studies, which suggests variability in tolerance among *R. sylvatica* populations. The deicers varied greatly in their toxicity, and further research is warranted to examine the differential effects of this suite of deicers on other species. Environ. Toxicol. Chem. 2011;30:1637–1641. © 2011 SETAC

**Keywords**—Transportation Tadpoles *Lithobates sylvatica* Highways Ecotoxicology

## INTRODUCTION

In cold climates, a myriad of chemicals may be used to remove or prevent the formation of ice on roads, highways, sidewalks, and runways. The most common deicing chemical used on roads is sodium chloride (NaCl) because of its low cost and widespread availability. In the United States, an amount of NaCl estimated at 10 million metric tons is used each year [1], ranging from 0.3 to 17.6 metric tons per lane mile across 26 states [2]. Roads in the State of Michigan receive more NaCl per lane mile (12.9 metric tons) than any other Great Lakes state [2].

The intensive and widespread application of NaCl on an annual basis over the past few decades has resulted in an increase in the salinity of ground and surface waters in North America [3–7]. Anthropogenic sources of NaCl have been shown to affect ground and surface water quality negatively [8–11]. Direct impacts of NaCl contamination in surface waters arise primarily from increased chloride concentrations, changes in water density gradients, salt-induced stratification, and salt stimulation of algal growth, leading to eutrophication [7]. Because of the threat NaCl poses to human health and the aquatic environment, Environment Canada identified road deicing chemicals as toxic [10].

Because of the known negative environmental impacts of NaCl, numerous alternatives are currently being evaluated to improve deicing operations and reduce the use of deicing chemicals. State and municipal transportation agencies are evaluating these alternatives in an effort to maintain safe winter driving conditions while avoiding the environmental degradation and potential harm to aquatic life caused by NaCl [1]. These chemicals include different inorganic salts that may be used separately or in conjunction with NaCl (e.g., $CaCl_2$, $MgCl_2$, and KCl) [10], sodium formate ($CHNaO_2$), calcium magnesium acetate ([CMA], $C_8H_{12}CaMgO_8$), magnesium acetate ($C_4H_6MgO_4$), calcium acetate ($C_4H_6CaO_4$), glycol liquids, urea ($NH_4CO$) [12], methanol ($CH_3OH$) [13], tetra potassium pyrophosphate [14], and Ice Shear$^{TM}$ (an equimolar mixture of sodium acetate and sodium formate) [15]. Acetate chemicals, in particular, are often viewed as an environmentally friendly alternative to inorganic salts because they do not contain chloride [7]. Furthermore, not all chemical deicers are equally effective at deicing and may require the application of greater quantities to achieve the same results [1,16]. For example, $NH_4CO$, $CaCl_2$, and CMA require up to 1.2 to 1.7 times as much deicer to achieve the same deicing result as NaCl [1]. This higher application rate could exacerbate negative environmental impacts of these chemicals. Because all of these chemicals differ in their chemical makeup and expected environmental concentrations (resulting from differences in application, mobility, and decomposition rates), extensive testing of their ecotoxicological effects should predate their widespread use.

Few studies have addressed the effects of NaCl on wildlife species, but limited research has shown that road salt exposure negatively affects mammals, birds, invertebrates, and amphibians that utilize roadside habitats [7]. Among these taxa, amphibians are likely to be the most affected by chemical deicer runoff. Amphibians possess highly permeable skin and have aquatic larval stages, and many use roadside wetlands for breeding [17]. Embryonic and larval amphibians exposed to salinities beyond their natural range experience substantial negative impacts. For example, high salinity may decrease development rate and increase malformations in embryonic and larval amphibians [18–21]. In addition, exposure to NaCl can increase infection rate of a lethal water mold in embryonic amphibians [22]. Furthermore, amphibians experience elevated

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Published online 6 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

levels of NaCl contamination during spring melt and runoff, and many species breed in small, ephemeral pools where NaCl runoff is likely to be concentrated [20,21]. As such, amphibians are considered indicators of ecosystem health and are model organisms for investigating the environmental effects of contamination from NaCl and other chemical deicers. The lethal and sublethal effects of NaCl contamination on amphibians have been addressed in a studies, which suggest that amphibians are negatively impacted at environmentally realistic concentrations [21,23,24]. In addition, previous research suggests that frogs and salamanders avoid salt-polluted pools and have not demonstrated local adaptation to high salinities when using roadside water bodies [21,25]. Furthermore, amphibian species may differ in their response to exposure to chemical deicers in runoff, which is predicted to influence demography and community structure [26].

Little is known about the relative population and ecosystem impacts of road salt alternatives and additives within the deicers. One study has directly compared the response of larval amphibians to exposure of NaCl and alternative deicers. Dougherty and Smith [13] compared the lethal effects of NaCl and an alternative ($MgCl_2$) on two native amphibians, green frogs (*Rana* [*Lithobates*] *clamitans*) and American toads (*Bufo* [*Anaxyrus*] *americanus*). To our knowledge, no other studies have directly compared the relative lethal effects of NaCl and a suite of commonly proposed alternative deicers on North American amphibians. The objective of the present study was to assess the direct acute toxicity of six deicing chemicals to native *R. sylvatica* larvae as a predictor of their relative toxicity in the environment.

## MATERIALS AND METHODS

### *Experimental design*

A series of 96-h acute toxicity tests using *R. sylvatica* larvae was conducted to determine the lethal effects of exposure to the following six chemical deicers: urea (pelleted fertilizer, $CH_4N_2O$; Garner Brothers), sodium chloride (coarse rock salt, NaCl; Morton Salt), calcium chloride (pelleted, $CaCl_2$; Peladow®, Dow Chemical), magnesium chloride (anhydrous, $MgCl_2$; Schoenburg Salt), potassium acetate (liquid, KAc, $CH_3COOK$; Cryotech CF7® Liquid Commercial Deicer; Crytotech Deicing Technology), and calcium magnesium acetate (pelleted, CMA, $C_8H_{12}CaMgO_8$; Cryotech CMA®; Crytotech Deicing Technology).

On May 8, 2008, nine recently deposited *R. sylvatica* egg masses were collected from a palustrine wetland adjacent to a moderately traveled road in Baraga County, Michigan (latitude 46.796 N, longitude 88.390 W). This road receives a low amount of salt (2.74 tons per lane mile for the winter of 2007–2008) during winter maintenance activities (D.J. Mills, Baraga County Road Commission, personal communication). The egg masses were transported to the laboratory and randomly assigned to one of four aerated 78-L glass aquaria containing approximately 50 L of filtered water from Portage Lake, Houghton County, Michigan. The eggs began hatching 5 d later. The tadpoles were fed ad libitum a 3:1 mixture of TetraFin flake goldfish food (Tetra Werke) and pulverized Purina rabbit chow (Purina Mills) from the time they hatched until they were placed into the test chambers. Larvae in test chambers were not fed during the experiment.

Methods for the 96-h acute toxicity tests strictly followed the protocols set forth by the American Society of Testing Materials (ASTM) [27] and the U.S. Environmental Protection Agency (U.S. EPA) [28] for static toxicity tests. The range of nominal test concentrations of chemical deicers in this experiment was chosen to encompass both known median lethal concentrations ($LC50_{96\text{-h}}$) for NaCl exposure to larval *R. sylvatica* and environmental concentrations of $Cl^-$ in wetlands and vernal pools resulting from NaCl pollution (0.002–10.3 $g\,L^{-1}$) [10,13,20,21,24,25,29]. Test chambers consisted of 44 glass jars with loosely fitting glass lids to prevent evaporation and allow for sufficient oxygen exchange. Two liters of filtered Portage Lake water and the appropriate amount of chemical deicer were added to obtain the following 11 nominal test concentrations: 0 (negative control), 0.19, 0.32, 0.54, 0.90, 1.50, 2.40, 3.84, 6.14, 9.83, and 15.73 $g\,L^{-1}$. The 11 nominal test concentrations were replicated four times, for a total of 44 experimental units per deicer.

Deicer treatments were randomly assigned to each jar. The treatment solutions were mixed until the chemical deicer was completely dissolved in each jar. Tadpoles were pooled from all egg masses and randomly assigned tadpoles of similar size to each test chamber (experimental units). Each experimental unit contained 10 tadpoles, except for $CaCl_2$ treatments, which contained five tadpoles per replicate because of a limited supply of larvae. Test chambers were maintained in the laboratory on a 12:12-h light:dark cycle using full-spectrum lights. Water temperature averaged 20.7°C (range 19.4–21.8°C) during all trials. Larvae were checked every 24 h, with mortality recorded at each interval. Larvae that were dead or unresponsive to probing with a small net were removed from the jar and preserved in a solution of 10% formalin. After 96 h, all tadpoles remaining in the test chambers were preserved. From these data, the LC50 value was estimated using the methods described below.

### *Statistical analysis*

Because survival data were not normally distributed, nonparametric statistics were used to examine differences in survival across test concentrations. For each chemical deicer, the proportion of larvae surviving at 96 h among treatments was analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance test. To determine the lowest concentration that had significantly lower survival than in the control, we used a Kruskal–Wallis test with multiple post hoc comparisons. The trimmed Spearman–Karber program (version 1.5) was used to calculate the LC50 estimates using untransformed data for each chemical deicer at 24, 48, 72, and 96 h of exposure [30,31]. The survival data were pooled for each concentration across replicates when calculating the LC50 value. The program R: A Language and Environment for Statistical Computing was used to perform all statistical analyses with an $\alpha$ level of 0.05 [32].

## RESULTS

Survival of *R. sylvatica* tadpoles after 96 h of exposure varied widely across deicers and concentrations (Fig. 1). Survival was 100% in all the control tanks except for the one assigned to the $CH_4N_2O$ treatments, and in this control survival was 95%. A significant effect of concentration on survival was detected for each deicer ($p = 0.038$). Tadpole survival was significantly lower in concentrations of $CH_4N_2O$ at 9.83 $g\,L^{-1}$ or higher compared with the control. Exposure to NaCl and $MgCl_2$ concentrations of 6.14 $g\,L^{-1}$ or above significantly reduced tadpole survival compared with the control. For $CH_3COOK$ and $CaCl_2$, 3.84 $g\,L^{-1}$ was the lowest concentration to cause significantly lower survival than in the control.

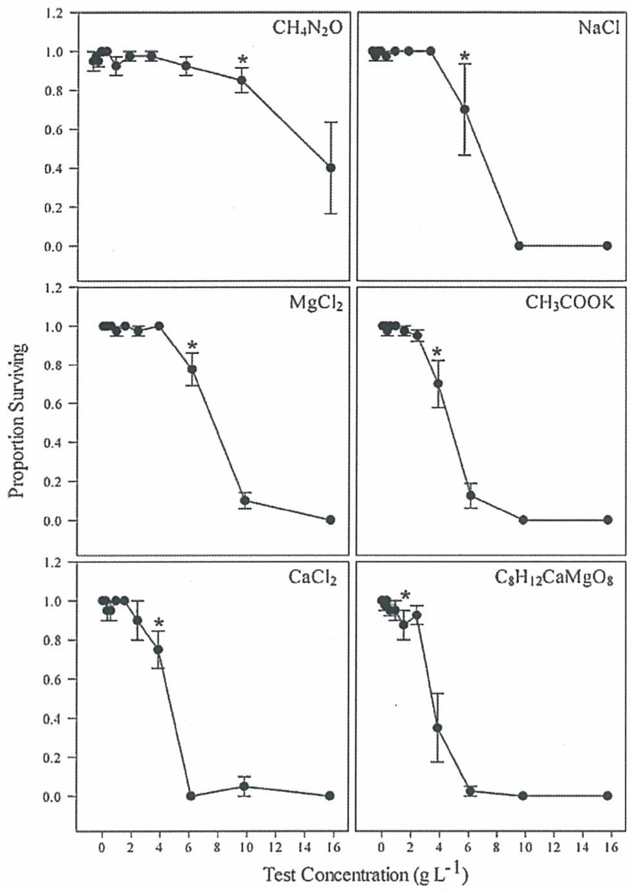


Fig. 1. Mean proportion of surviving *Rana sylvatica* tadpoles after 96 h of exposure to 10 nominal test concentrations of six chemical deicers. Error bars represent ± standard error. Asterisks indicate the lowest test concentration at which survival was significantly lower than in the control ($n = 4$ replicates per concentration).

Exposure to concentrations of $C_8H_{12}CaMgO_8$ at or above 1.50 g $L^{-1}$ caused significantly lower survival. Survival in test concentrations below those presented above for each deicer was not significantly lower than that in the control ($p > 0.05$).

Different toxicities of the six chemical deicers suggested by tadpole mortality were supported by estimated $LC50_{96\text{-h}}$ values, which ranged from 3.23 g $L^{-1}$ ($C_8H_{12}CaMgO_8$) to 14.63 g $L^{-1}$ ($CH_4N_2O$; Table 1). The estimated $LC50_{96\text{-h}}$ values were highest for $CH_4N_2O$, NaCl, and $MgCl_2$ and were much lower for acetate chemicals ($C_8H_{12}CaMgO_8$, $CH_3COOK$) and $CaCl_2$. For each chemical, the LC50 values were highest after 24 h of exposure and decreased by 2 to 40% through time until the end of the trial.

## DISCUSSION

Survival of *R. sylvatica* larvae was reduced by exposure to higher concentrations of all chemical deicers examined in this study; however, the response by larvae depended on the chemical deicer. In general, urea and several of the chloride compounds were less toxic than acetates ($C_8H_{12}CaMgO_8$, $CH_3COOK$) and $CaCl_2$. At each time in the test, $C_8H_{12}CaMgO_8$ exposure induced 50% mortality at the lowest concentration compared with all other chemicals. In addition, LC50 values decreased with time, indicating either that larval *R. sylvatica* were less able to tolerate or offset the physiological or toxic stress associated with these compounds as duration of exposure increased or that there was a lag in the lethal effects of initial exposure. If the duration of exposure was critical, this suggests that the effects of exposure to winter season road maintenance involving deicers depend on the type of chemical applied, the concentration that builds up in the environment, and how long the chemical persists in the environment. If there was a lag response in mortality, then possibly even short-term exposure to the contamination could have lethal effects on amphibian larvae.

We are not aware of previous studies on amphibians that have investigated the toxicity of urea in the context of its use as an alternative chemical deicer. Urea is widely used in the United States as a source of fertilizer for both agricultural and forest lands as well as an aircraft deicer. Adult amphibians avoid water bodies that receive urea runoff, even when concentrations are lower than the recommended terrestrial fertilization rates [33]. In this study, the high tolerance of *R. sylvatica* tadpoles for urea compared with the other five chemical deicers is expected given that tadpoles excrete urea as a waste product and may retain urea as an osmolyte to protect against salt stress and dehydration [19]. In addition, urea may be used a cryoprotectant by amphibians exposed to low temperatures and freeze–thaw cycles, as is likely during the early breeding cycle of *R. sylvatica* [34]. However, when exposed to high concentrations of urea in their environment, amphibians experience deleterious effects on protein structure and function [35]. Results of the present study suggest that *R. sylvatica* larvae appear to tolerate relatively high concentrations (<9.8 g $L^{-1}$) of urea during short-term exposure.

The $LC50_{96\text{-h}}$ values estimated in the present study are over two times greater than values reported in previous studies with *R. sylvatica* (Table 2) [23,24]. The discrepancy between this and other studies suggests that this population may be more tolerant to short-term exposure to NaCl pollution than are other populations. The larvae tested in this experiment were collected adjacent to a road that receives a small amount of NaCl, with sand as the primary winter maintenance product (4–5% NaCl; D.J. Mills, personal communication). It is possible that localized adaptation or acquired tolerance to NaCl pollution could solely explain this difference in tolerance, although this phenomenon has not been previously documented in *R. sylvatica* or spotted salamanders (*Ambystoma maculatum*) [20,25].

In addition, differences in experimental methodology and adherence to ASTM or U.S. EPA guidelines may confound comparisons between toxicity studies on chemical deicers with amphibians. The use of food-grade salt, alternative methods of statistical analysis [24], purified or deionized water [13,21,24], and plastic [21] or glass containers may affect resultant

Table 1. Median lethal concentration values (LC50, g $L^{-1}$) with their 95% confidence limits for larval *Rana sylvatica* at 24, 48, 72, and 96 h of exposure to six chemical deicers during acute toxicity tests ($n = 4$)

| Value | $CH_4N_2O$ | NaCl | $MgCl_2$ | $CH_3COOK$ | $CaCl_2$ | $C_8H_{12}CaMgO_8$ |
|---|---|---|---|---|---|---|
| $LC50_{24\text{-h}}$ | 14.63 (12.83-16.69) | 9.12 (8.53-9.82) | 7.37 (6.81-7.99) | 7.03 (6.22-7.95) | 4.85 (4.16-5.65) | 3.43 (3.13-3.81) |
| $LC50_{48\text{-h}}$ | 14.37 (12.77-16.18) | 7.82 (7.64-8.01) | 7.28 (6.73-7.92) | 5.42 (4.85-6.06) | 4.72 (4.08-5.47) | 3.39 (3.07-3.74) |
| $LC50_{72\text{-h}}$ | 14.37 (12.77-16.18) | 7.64 (7.46-7.82) | 7.24 (6.64-7.82) | 4.76 (4.27-5.31) | 4.18 (3.69-4.73) | 3.31 (2.97-3.68) |
| $LC50_{96\text{-h}}$ | 14.29 (12.55-16.26) | 7.56 (7.31-7.82) | 7.11 (6.54-7.74) | 4.23 (3.84-4.66) | 3.98 (3.46-4.57) | 3.23 (2.94-3.59) |

Table 2. Median lethal concentration values ($LC50_{96\text{-h}}$, $g\ L^{-1}$) and 95% confidence limits where available for larval amphibians native to the northern and eastern United States during acute exposure to deicing chemicals

| Species | Deicer | LC50 |
|---|---|---|
| *Ambystoma maculatum* | NaCl | 1.84 (1.42-2.39)[a] |
| *Bufo americanus* | NaCl | 6.14 (5.94-6.48)[a] |
| | $MgCl_2$ | 0.105[b] |
| *Hyla versicolor* | NaCl | 1.05[c] |
| *Pseudacris crucifer* | NaCl | 4.43 (3.89-5.05)[a] |
| *Rana clamitans* | NaCl | 0.406[b] |
| | | 4.86 (4.42-5.36)[a] |
| | $MgCl_2$ | 0.116[b] |
| *Rana sylvatica* | NaCl | 2.64 (2.53-2.74)[d,*] |
| | | 2.69 (2.31-3.14)[a] |
| | | 5.11 (5.46-6.93)[d,**] |
| | $MgCl_2$ | 0.23[b] |
| | $Ca(CH_3CO_2)_2$ | 0.48[b] |
| | $Mg(CH_3CO_2)_2$ | 6.61[b] |
| | $Na_4Fe(CN)_6$ | 2.06[b] |

[a] Collins and Russell [23].
[b] Dougherty and Smith [13].
[c] Brand et al. [37].
[d] Sanzo and Hecnar [24].
[*] Calculated using Spearman-Karber Analysis.
[**] Calculated using Probit Analysis.

$LC50_{96\text{-h}}$ values. Also, feeding tadpoles during exposure and conducting acute toxicity tests at a different room temperature may also influence results [24]. We expect that the use of coarse (nonpurified) rock salt in winter maintenance, a lack of food availability, and the use of glass in experimental chambers in this study (as outlined in ASTM guidelines) would have produced different $LC50_{96\text{-h}}$ values compared with previous studies. Plastic containers might have an interactive effect and interfere with estimates of toxicity endpoints if they leach additional chemicals. For example, plasticizers such as bisphenol A and dibutyl phthalate may cause adverse effects on embryonic or larval amphibians, including malformations, early mortality, and sex reversal [36]. Strict standardization of experimental protocol is recommended in future toxicity tests to facilitate comparisons between studies on different populations or species.

Magnesium chloride and $CaCl_2$ are used primarily as fugitive dust inhibitors on unpaved roadways and to a lesser degree as chemical deicers; however, they are commonly available in stores as consumer-level sidewalk deicers. As a chemical deicer, these are slightly more efficient than NaCl in removing ice. Under the same application rates as NaCl, $MgCl_2$ and $CaCl_2$ application will contribute more detrimental $Cl^-$ into the roadside environment, further expounding the negative impacts of chemical deicer application [1]. This raises serious concerns over the choice of either of these chemicals as an alternative to NaCl. In addition, our results and those of Dougherty and Smith [13] suggest that native larval amphibians are much more sensitive to both $MgCl_2$ and $CaCl_2$ than to NaCl.

Variation in salt tolerance among North American amphibians has been described elsewhere (Table 2). Embryonic and larval *R. clamitans* tadpoles were found to be relatively insensitive to NaCl pollution, with low mortality rates [21] and moderate $LC50_{96\text{-h}}$ values [23]. However, Dougherty and Smith [13] found larval *R. clamitans* to be intolerant of NaCl pollution. Larval gray tree frogs (*Hyla versicolor*) [37] and *A. maculatum* [23] were also reported to be less tolerant of NaCl pollution than other native species. Larval *R. sylvatica* and spring peepers (*Pseudacris crucifer*) appear to be moderately tolerant of NaCl pollution, with low $LC50_{96\text{-h}}$ values [23]. *Bufo americanus* larvae were most tolerant of NaCl pollution [23] and had 100% survival in acute exposure up to $3.0\,g\,L^{-1}$ [13]. This tolerance exhibited by *Bufo* may stem from past selection to tolerate extreme and rapid drought conditions that could lead to rapid rises in solute concentrations. Different salt tolerance levels among species may influence demography and community structure of native amphibians, particularly for those using roadside breeding habitats [23,26].

To our knowledge, no other studies have investigated the acute effects of the range of NaCl alternatives on native amphibians. One study has investigated the acute toxicity of NaCl and an alternative deicer on amphibians. Results of Dougherty and Smith [13] suggest that *B. americanus*, *R. clamitans*, and *R. sylvatica* are much more sensitive to $MgCl_2$ exposure than NaCl pollution, with $LC50_{96\text{-h}}$ values ranging from 0.11 to $0.23\,g\,L^{-1}$ (Table 2). These estimates for $MgCl_2$ exposure are also much lower than those estimated in this study, suggesting that this population may be more tolerant of exposure than other populations. Knowledge of the $MgCl_2$ tolerance of other groups of native amphibians will help to determine the effect of this pollution source on native amphibian communities. The identification of the environmental effects of alternative chemicals to aquatic and terrestrial organisms is essential prior to implementation of these chemicals as a viable alternative to NaCl.

Knowledge of the potential and quantified environmental impacts of the other alternative deicing chemicals used in this study is limited. This is particularly important to consider for acetate chemicals, because they are generally considered an environmentally friendly alternative to NaCl [7]. The behaviour of $C_8H_{12}CaMgO_8$ and $CH_3COOK$ in the environment raises serious concerns about potential widespread use of these chemicals as a winter maintenance tool. When CMA is used as road deicer, average highway spray and runoff concentrations of CMA would likely range from 10 to 100 mg/L, with average annual loadings of 10 tons per mile [38]. In surface water, CMA (acetate) decomposition is predicted to occur in 100 d at 2°C and much faster at higher water temperatures [38]. Acetate products may also decrease the pH of roadside soils and lead to the mobilization of heavy metals. In aquatic environments, acetate products increase oxygen demand and may decrease the biomass of algae [1,16], which is a common food resource for developing larval amphibians. These potential environmental effects of $C_8H_{12}CaMgO_8$ and $CH_3COOK$ may have grave implications for sensitive embryonic and larval amphibians by reducing the availability of oxygen and algae necessary for proper development. Results of this study showing low $LC50_{96\text{-h}}$ values for $C_8H_{12}CaMgO_8$ ($3.23\,g\,L^{-1}$) and $CH_3COOK$ ($4.23\,g\,L^{-1}$) exposure demonstrates that this alternative deicer may indeed be more harmful than road salt and other deicing chemicals to amphibian communities.

Although the median lethal estimates of chemical deicers to amphibians in this study are above environmentally realistic concentrations of residual chloride from NaCl application in roadside water bodies, we cannot assume a lack of adverse effects of these chemicals on amphibians. The U.S. EPA categorizes substances with an LC50 above $0.10\,g\,L^{-1}$ to be practically nontoxic to aquatic organisms (http://www.epa.gov/espp/litstatus/effects/redleg-frog/naled/appendix-i.pdf). Similarly, Environment Canada considers prolonged exposure to $Cl^-$ concentrations above $0.220\,g\,L^{-1}$ as harmful to approximately 10% of aquatic species [10]. However, previous studies indicate NaCl concentrations as low as $0.078\,g\,L^{-1}$ can cause significant sublethal effects, including decreased survival over time, decreased number of frogs that metamorphose, and delayed time to metamorphosis in *R. sylvatica* [24]. Residual chloride concentrations in roadside water bodies range from 0.002 to 10.3 $g L^{-1}$ and are highest in early spring and late summer [21,23,24]. Considering that inorganic salts other than NaCl used as chemical deicers will likely contribute more $Cl^-$ into the environment, we can expect residual chloride levels to be higher in freshwater systems adjacent to roadways receiving $MgCl_2$ and $CaCl_2$. In addition, the higher application rate required for effective winter maintenance using $C_8H_{12}CaMgO_8$ and $CH_3COOK$ suggests that the concentrations of these chemicals in roadside water bodies will be close to or above the LC50 estimates for amphibians.

The effects of chemical deicers in the environment depend on the rate at which they are applied and their persistence in the environment. Road salt alternatives may cause greater environmental degradation, because, relative to NaCl, greater quantities have to be applied to achieve similar levels of road deicing [1,28]. Thus, the negative impacts of these chemicals on amphibian communities likely will be elevated. Future work on lessening the negative impacts of NaCl should focus on the application of reduced amounts of NaCl or nonchemical approaches instead of relying on alternative chemical deicers.

*Acknowledgement*—We are indebted to R. Alger, P. Nankervis, M. Mitchell, H. Erickson, and E. Rogers for assistance in both field and laboratory portions of the experiment. We thank three anonymous reviewers for their thorough review of the manuscript and helpful suggestions. Funding for this research was provided by grants from the DeVlieg Foundation, the Chicago Herpetological Society, the Western New York Herpetological Society, the Amphibian Specialist Group of the International Union for Conservation of Nature and Natural Resources, the Ecosystem Science Center at Michigan Technological University, with support from the MTU Global Watershed GK-12 program to M. Harless. This research has also been supported by funding and infrastructure provided by Michigan Technological University. Embryos were collected under permissions granted by the Keweenaw Bay Indian Community Department of Natural Resources. We conducted laboratory research under permit by Michigan Technological University (IUCAC permit L01401).

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# *KBIC L'Anse Reservation Water Chemistry Study Results* 2009-2010

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## I. Background

Pollution from road runoff often includes a wide array of chemicals, such as hydrocarbons and heavy metals from vehicles (Transportation Research Board 1991), and a separate assortment of contaminants resulting from winter road-clearing operations involving the removal and prevention of ice (Gales and VanderMeulen 1992; Paschka et al. 1999). The most commonly applied deicing and anti-icing chemical is road salt in the form of sodium chloride (NaCl). An estimated 14 million tons of road salt are annually deposited on North American roads (Transportation Research Board 1991; Environment Canada 2001), representing a significant source of environmental pollution with major implications for ecosystems and the biological life they support. Widespread contamination of freshwater habitats and groundwater sources from road salt deposition is well documented, including changes increased chloride levels, salt-induced stratification of water columns, and eutrophication (*reviewed in* Ramakrishna and Viraraghavan 2005). Elevated chloride levels are considered a major stressor to freshwater organisms and may put aquatic communities at risk within the next century (Kaushal et al. 2005; Karraker 2007). Even brief exposure to high chloride concentrations is potentially very harmful to sensitive wildlife species.

Amphibians are particularly sensitive to chemical contaminants due to their highly permeable skin, aquatic larval stages, and use of roadside wetlands for breeding (Stebbins and Cohen 1995). As such, amphibians are considered effective indicators of ecosystem health and are model organisms to investigate the effects of road salt contamination. Nonetheless, the direct effects of road salt on amphibian species have received little attention (e.g., Turtle 2000; Sanzo and Hecnar 2006; Karraker 2007). Turtle (2000) observed lower survivorship of spotted salamanders (*Ambystoma maculatum*) in roadside pools contaminated by road salt than in woodland ponds. After acute and chronic exposure to road salt, larval wood frogs (*Rana sylvatica*) experienced stress, increased mortality, and altered development (Sanzo and Hecnar 2006).

Preliminary results of our ongoing laboratory studies on the effects of acute and chronic exposure to a suite of deicers (including road salt) support published results and suggest negative implications for northern populations of wood frogs and green frogs (*Rana clamitans*; Harless et al. 2011). Karraker (2007) found that embryonic and larval stages of *A. maculatum* and *R. sylvatica* showed increased mortality and frequency of malformations during development when exposed to concentrations of salts observed in some areas. Clearly, freshwater contamination by road salt poses a serious threat to amphibian survival, and may be contributing to widespread population declines observed in more northern latitudes. These studies identify a need to further examine the effect of chemical deicers on amphibians in an ecological context.

While the application of road salt in the local area (range: 3.2-4.6 tons per lane mile; D.J. Mills, pers. comm.) is below the state average (12.9 tons per lane mile; Transportation Research Board 1991), information concerning the salt tolerance of local amphibians is useful in understanding the effects of this pollution source on this sensitive group of organisms. This information will be useful regarding the population level responses of amphibian populations across the United States Furthermore, investigating the spatial relationship between this lower level of road salt application and the residual chloride levels in local water bodies will help to identify how this deposition is affecting amphibian habitats in cold climates.

As a part of my dissertation research project at Michigan Technological University, I initiated a broad scale survey of water chemistry data in 130 local wetlands and vernal pools in 2009. My research project focuses on the impact of road salt (NaCl) on amphibian communities. We utilize a mixture of laboratory experiments and field surveys to identify both the short and long term impacts of exposure to road salt on native amphibian larvae. Our laboratory studies help us to identify the lethal and sublethal levels of road salt exposure to amphibians whereas our field surveys allow us to identify local amphibian habitats that may be potential harmful to breeding amphibians.

This report focuses on the results of the water chemistry data collection and analysis that took place on the Keweenaw Bay Indian Community L'Anse Reservation. Below we provide the location of the wetlands sampled, the water chemistry measurements from those water bodies, and an examination of these sites in a regional context.

## II. Methods

To determine the threat local amphibians face when utilizing roadside habitats for breeding, we visited 36 wetlands on the KBIC L'Anse Reservation in 2009 and 2010 (Table 1). In 2009, these wetlands were sampled as a part of a broad scale survey of 130 wetlands in Baraga, Keweenaw, and Houghton Counties. Thus, we visited wetlands at different intervals in 2009. In 2010, we focused our efforts on a strict biweekly sampling scheme of 10 wetlands and vernal pools each in Baraga and Houghton Counties.

At each visit we recorded pH, conductivity, salinity, and water temperature in each wetland or vernal pool using a YSI 63 Multimeter probe. We also collected a sample of the water for use in ion chromatography analysis for the determination of chloride ion [$Cl^-$] concentration using a Dionex Ion Chromatography machine we were able to use in collaboration with the Environmental Engineering department at MTU. Chloride ions remain in surface water after road salt application and can be used to estimate the extent of recent road salt deposition (Karraker 2007*a*; Findlay and Kelly 2011). We estimated the chloride concentrations in samples from 2009. We have not yet completed the 2010 samples. To examine the spatial relationship between the location of the nearest salt treated highway and the water body, we used ArcGIS to measure the shortest distance between the sample location and the nearest state highway.

## III. Results

Results suggest that water chemistry values in KBIC wetlands varied throughout the spring, summer, and fall (Figure 1). However, these differences were not significant for each of the three measurements. For pH, we observed the lowest values in early spring and late fall in 2009. In 2010, the lowest pH values occurred in early spring and mid-summer. Furthermore, mean conductivity levels did not vary significantly through the breeding season in 2009 nor 2010. These levels were variable across all months in 2009 whereas with our strict sampling regime in 2010 we observed bell shaped pattern with conductivity peaking in July and August. Trends in salinity measurements on the KBIC property mirrored those of the conductivity values as the YSI probe estimates salinity using the measured conductivity value. Henceforth, we will focus our examination of salt tolerance focusing on the conductivity measurements and ignoring these salinity values.

In examining the spatial relationship between road salt application on local managed highways and chloride concentrations across the three counties, we observed an exponential decrease in chloride concentration as distance from the road increased (Figure 2). However, this difference was not significant. The highest chloride concentrations were observed in water bodies within 1000m of a salt-treated highway. Chloride concentration estimates from the 2009 samples were variable through the breeding season and peaked in July and August (Figure 3). There was no significant trend in chloride concentrations over time in these water bodies.

Table 1. Physical attributes of study sites used in collecting water chemistry data on the KBIC L'Anse Reservation. Distance from nearest paved road and salt-treated highway were calculated using ArcGIS Software.

| *Site Name* | *Easting* | *Northing* | *Distance to Nearest Paved Road (m)* | *Distance to Nearest Highway (m)* |
|---|---|---|---|---|
| Third Lake | 394664 | 5180950 | 342.20 | 5425.68 |
| Arvon RD S | 397522 | 5178933 | 1677.32 | 6776.65 |
| Arvon Road* | 398041 | 5179044 | 2309.14 | 7358.73 |
| Baily Lake Wetland | 415208 | 5256695 | 44.43 | 44.43 |
| Baraga 1 | 385758 | 5182235 | 67.63 | 477.69 |
| Baraga 2 | 385425 | 5182369 | 205.09 | 591.42 |
| Baraga Village | 387004 | 5182545 | 8.43 | 170.50 |
| Beartown Corners | 384083 | 5185028 | 821.74 | 3184.62 |
| Beesley Corner | 401002 | 5189209 | 2.83 | 15547.32 |
| Hatchery Ponds | 394760 | 5188607 | 13.46 | 11331.63 |
| Heltunen Road | 399415 | 5189723 | 704.78 | 14831.20 |
| Herman Road | 393307 | 5171719 | 2.65 | 3767.71 |
| Indian Rd* | 392863 | 5172461 | 70.20 | 3087.47 |
| Kelsey Creek | 382617 | 5187437 | 1882.67 | 4027.87 |
| Kelsey Creek West | 382751 | 5187597 | 2065.49 | 3944.73 |
| Laugh's Lake | 396004 | 5176713 | 696.61 | 5085.48 |
| Mud Lakes North* | 386868 | 5186355 | 135.72 | 135.72 |
| Mud Lakes South | 387158 | 5185951 | 3.57 | 93.58 |
| Pequaming Bog East* | 395831 | 5190099 | 21.51 | 13164.84 |
| Pequaming Bog West | 394381 | 5189320 | 25.74 | 11838.41 |
| Pikes Peak* | 398603 | 5189147 | 151.89 | 13849.79 |
| Pinery Lakes | 393580 | 5180190 | 812.45 | 3958.64 |
| Sand Point | 388140 | 5182742 | 686.73 | 686.73 |
| Sand Point NE | 388187 | 5182636 | 768.22 | 768.22 |
| Sand Point SW | 387688 | 5182106 | 624.62 | 624.62 |
| Silver Road | 401817 | 5179998 | 4658.37 | 9106.65 |
| Silver Road 1 | 401817 | 5179998 | 6160.49 | 11312.09 |
| Silver Road 2 | 402777 | 5176917 | 7089.35 | 11797.14 |
| Silver Road 3 | 403724 | 5176917 | 7939.35 | 12749.19 |
| Skanee Marsh* | 394534 | 5183373 | 50.08 | 6918.47 |
| Skanee Marsh 1 | 394611 | 5183475 | 9.83 | 7006.07 |
| US 41 L* | 389313 | 5172360 | 43.09 | 43.09 |
| US 41 R* | 389094 | 5172131 | 36.85 | 36.85 |
| US41 Dump * | 385797 | 5178916 | 9.48 | 9.48 |

* = sites sampled during both 2009 and 2010.

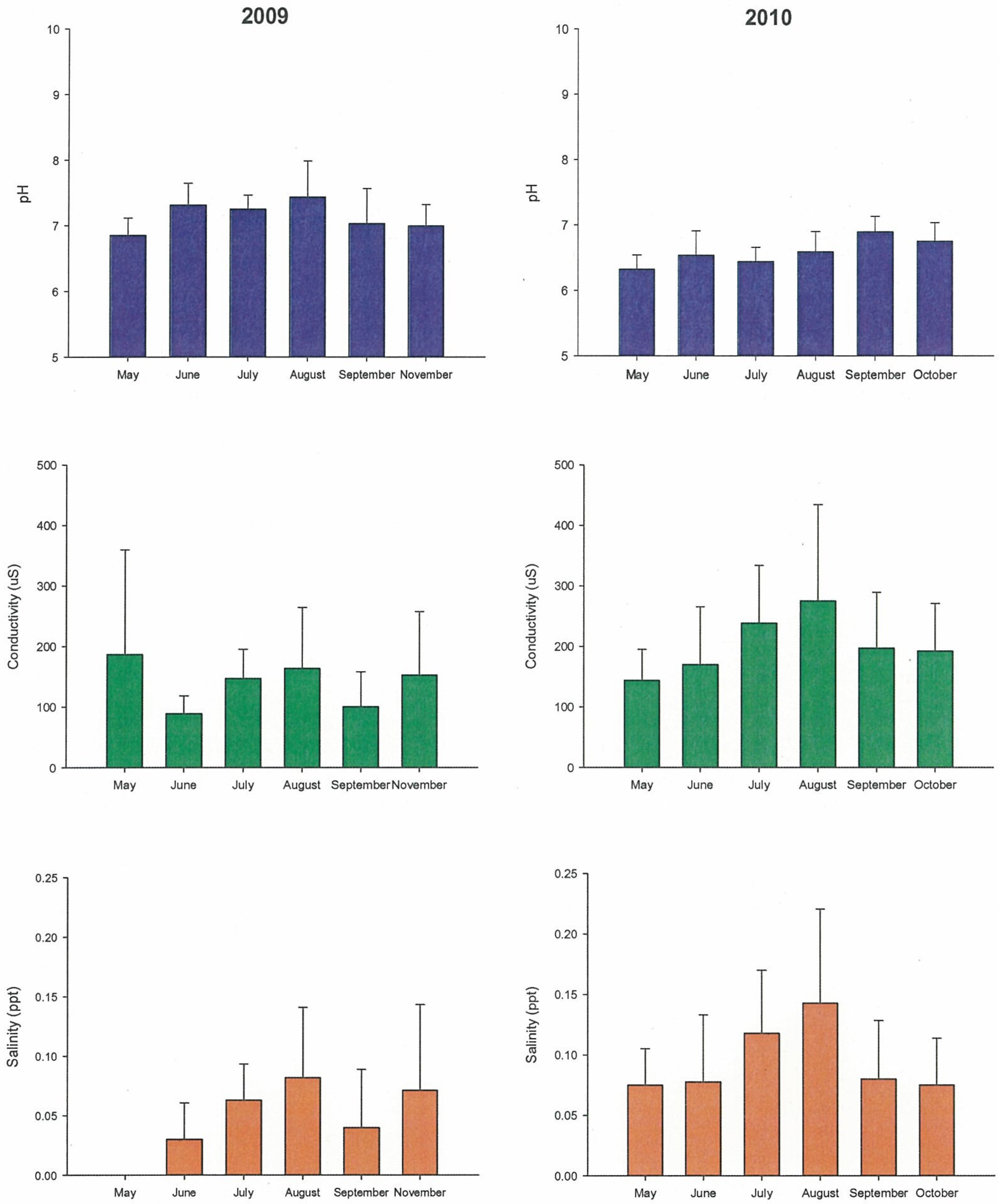


Figure 1. Mean monthly values for pH, conductivity (μS), and salinity (ppt) in KBIC wetlands and vernal pools for 2009 and 2010. Error bars represent 2 standard error.

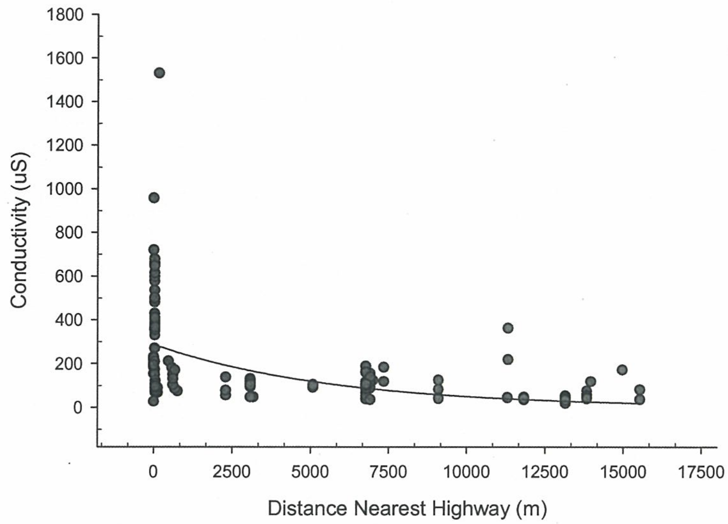


Figure 2. Relationship between conductivity (μS) and distance from the nearest road salt treated highway (m) from water samples collected from water bodies in Baraga, Houghton, and Keweenaw counties over 2009 and 2010. The trendline represents a polynomial fit curve.

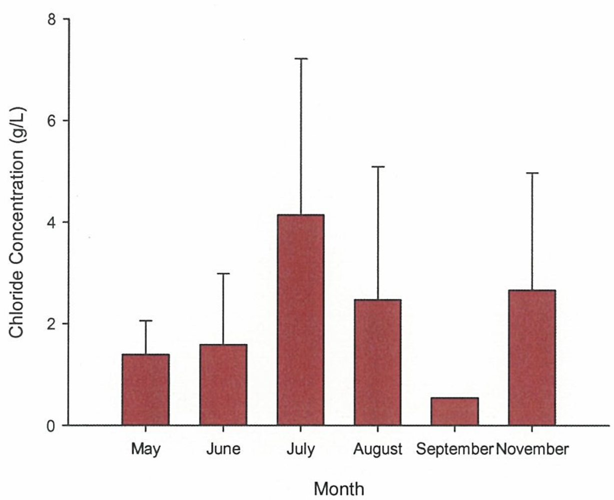


Figure 3. Mean monthly estimates of chloride concentration ($gL^{-1}$) from ion chromatography analysis in water samples from KBIC wetlands and vernal pools in 2009.

## IV. Discussion

The variation in pH through the amphibian breeding season suggests that low pH may occur as a result of low precipitation inputs or high evaporation in local wetlands and vernal pools. Low pH values are known to have negative impacts on larval amphibians such as increasing time to metamorphosis and mortality (Glos et al. 2003). The pH values in this survey ranged from 6.2 – 8.1 over the entire breeding season, suggesting that amphibians breeding in wetlands on the KBIC were not at risk of exposure to harmful acidic habitats.

Conductivity is often used as a surrogate for salt in field studies with amphibians. Karraker (2007) observed a significant reduction in embryonic and larval survival at conductivity levels above 500 µS in *Ambystoma maculatum* and 3000 µS for *Rana sylvatica*. Additionally, high incidences of malformations in larval R. sylvatica were observed (Karraker 2007). Sanzo and Hecnar (2006) observed a significant reduction in larval survival in *R. sylvatica* above 2000 µS. Given these tolerance levels, no sites sampled on the KBIC property exceeded these values during the sampling period. This suggests that the conductivity levels in these water bodies are not harmful to local amphibian populations. In examining the conductivity measurements from the broad scale survey of three local counties over both years, only sampling sites within 50 meters of a salt treated road exceeded these conductivity thresholds (Figure 2).

Other studies have observed different trends in chloride concentration in field sampling of amphibian breeding habitat. Collins and Russell (2009) found mean chloride levels to be highest in spring (0.118 g/L; range: 0.004-0.586), while increasing from early summer (0.082 g/L; range .004-.410) to late summer (0.097 g/L; range: 0.004-0.427). These results as well as ours suggest that chloride concentration increases in late summer when evaporation is highest in wetlands and vernal pools. Sanzo and Hecnar (2006) measured a range of chloride in wetlands near road salt treated roads between 0.004 and 10.3 g/L. Karraker observed a range of chloride levels from 0.145-0.945 g/L in vernal pools. Larval amphibians present in these water bodies may be exposed to potentially lethal levels of chloride.

Other studies on multiple amphibian species suggest that $LC_{50}$ values for chloride exposure range from 1.18 to 3.92 g/L (Sanzo and Hecnar 2006; Dougherty and Smith 2006; Collins and Russell 2009). Based on our analysis, water bodies across all months could potentially contain harmful chloride levels at these tolerance values. In addition, high chloride levels in July and August may be quite harmful to larval amphibians breeding in these wetlands on the KBIC property.

However, our preliminary data suggests that the $LC_{50}$ estimate for NaCl exposure may be higher than previously reported for *R. sylvatica* at 7.56 g/L (Harless et al. 2011). Further research on other populations of wood frogs will help to identify the tolerance of this species. Using our $LC_{50}$ levels, water bodies on the KBIC would contain harmful concentrations of chloride in July and August when water levels are low. Further analysis of samples from 2010 will help to shed light on this relationship.

In summary, the water chemistry analysis of the wetlands and vernal pools on the KBIC L'Anse Reservation suggests that these water bodies pose little threat to the survival and fitness of local breeding amphibians.

## V. Acknowledgements

We are graciously indebted to the Keweenaw Bay Indian Community for permissions granted to collect amphibian eggs, land access, and on-going project support. We thank Pam Nankervis for her helpful suggestions on the design of this study and her assistance in locating sampling sites. In addition, we thank Pam for her help in obtaining road salt estimates from the Baraga County Road Commission. Without her generosity, this research would not have been possible. We would also like to thank the following individuals for their unique contributions to the project: D. Perrum, A. Marcarelli, M. Mitchell, E. Gorsalitz, L. Turos, and D. Mundhal.

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